TOXICOLOGICAL EVALUATION OF DIETARY FUMONISIN B1 ON SERUM BIOCHEMISTRY OF GROWING PIGS

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ABSTRACT

Twenty-four male Large White weanling pigs of 8-9 weeks of age averaging 6.94±0.26 kg were used to evaluate the effect of dietary fumonisin B_1 (FB_1) on serum biochemical parameters. The animals were randomly assigned to 4 dietary treatments containing 0.2, 5.0, 10.0 and 15.0 mg FB_1/kg constituting the control, diets 1, 2 and 3 respectively, in a 6-month feeding trial. Blood sample was collected from the ear vein of each animal at the end of the feeding trial for biochemical analyses.

Animals fed the control diet and diet 1 had significantly ($P<0.05$) higher serum total protein, albumin and globulin concentrations as well as the serum alanine aminotransferase (ALT) activities and serum cholesterol concentrations than those on diets 2 and 3, while the serum triglyceride concentrations of the animals fed diets 1 and 2 were significantly ($P<0.05$) lower than those fed diet 3 but significantly ($P<0.05$) higher than those fed the control diet.

The study revealed that chronic ingestion of dietary $FB_1 \geq 10.0$ mg kg^{-1} may result in significant alterations of serum biochemical parameters in growing pigs suggesting chronic gastrointestinal or hepatic disease.

Key words: fumonisin B_1, serum biochemistry, growing pigs

INTRODUCTION

It has long been recognized by veterinarians that marked immunosuppression is observed in livestock ingesting mycotoxins at levels below those that cause overt toxicity [25]. Several reviews [6, 2] have detailed the effects of mycotoxins on immune response in laboratory animals. From an agricultural standpoint, mycotoxin–induced immunomodulation is significant as it is conceivable that altered immune function may contribute to the symptoms of some animal mycotoxicoses.

Fumonisins are mycotoxins produced by Fusarium molds, most notably Fusarium verticillioides (=F. moniliforme) and F. proliferatum. These mycotoxins occur as contaminants of agricultural products, particularly maize worldwide [21]. Shephard et al. [28] reported that maize, which is the major cereal utilized in the formulation of livestock feeds as well as a major dietary staple in several parts of the world, is the only commodity that contains significant amounts of fumonisins; hence the potential for fumonisins to be found in feeds and foodstuffs is high.

The major types of fumonisins are B_1, B_2 and B_3, and have been implicated in modification of immune response. The most prevalent of these mycotoxins as reported by Ross et al. [26] is fumonisin B_1 (FB_1). The facts that fumonisins cause field outbreaks of mycotoxicoses in animals, carcinogenic in rats (and probably human) [16], cause hepatic diseases in rats [13], disrupt sphingolipid metabolism [17] and lower nutrient utilization in growing pigs [10], have resulted in much recent interest in these compounds. Based on the reviewed physiological effects on animals, coupled with a survey of contemporary literature revealing increasing wave of fumonisin contamination of feeds and feedstuff [9], this study was designed to assess changes in serum biochemical profile of growing male Large White pigs fed diets with FB_1-contaminated maize.

MATERIALS AND METHODS

Fumonisin–contaminated maize grains, cultured with Fusarium verticillioides, were generated according to the method described by Nelson et al. [22] at the Plant Pathology Laboratory, International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. Three diets containing 5.0, 10.0 and 15.0 mg FB_1/kg constituting diets 1, 2 and 3, respectively, were formulated using ground Fusarium-cultured maize substituted for ground, autoclaved non-cultured maize in various proportions. With the control diet, containing approximately 0.2 mg FB_1/kg, the treatment diets were used in a 6-month feeding trial. The FB_1 concentrations were determined using the fumonisin qualitative test kit (Neogen Corp., USA)

Twenty-four male Large White weanling pigs (about 8 - 9 weeks of age) were assigned, in a completely randomized design, to each of the 4 dietary treatments, and 6 animals were used for each group. The feeding trial was divided into 3 physiological phases (weanling, pre-pubertal and pubertal). The gross composition of the treatment diets, fed during weanling, pre-pubertal and pubertal's phases for 6, 10 and 8 weeks, respectively, are shown in Table 1, and satisfied the nutrient requirements of the animals at the various physiological phases as recommended by

Table 1: Gross composition (%) of the test diets for the various physiological phases

	Physiological Phase		
Ingredient	Weanling	Pre-pubertal	Pubertal
*Maize	40.00	30.00	20.00
Soybean meal	20.00	15.00	8.50
Palm kernel cake	20.00	25.00	25.00
Wheat offal	14.00	14.30	5.00
Rice husk	-	11.00	17.80
Fish meal	3.00	2.00	1.00
**Fixed ingredients	2.70	2.70	2.70
Total	100.00	100.00	100.00
Analysed nutrients:			
Crude Fibre (%)	5.35	9.82	10.83
Crude Protein (%)	20.38	17.97	15.30
DE (Kcal/kg)	2701.80	2269.11	2240.61

*Mixture of *Fusarium*-cultured and non-cultured maize in various proportions to achieve desired dietary FB_1 levels for each treatment.

**Contained Dicalcium phosphate (1.50), Oyster shell (0.50), Salt (0.45) Minerals/Vitamins premix (0.20), Methionine (0.01) and Lysine (0.04).

NRC [19].

The animals were fed their respective diets ad libitum daily at 08:00 and 16:00hr. Cool, fresh and clean water was available ad libitum throughout the experimental period. At the beginning of the experiment and at the last day of each physiological phase, blood samples were collected from the ear vein of each animal into Monoject® vacutainer without Ethylene diaminetetraacetic acid (EDTA). Blood samples were centrifuged at 4000 rpm for 10 minutes and separated sera analysed for the determination of serum biochemical parameters at the Chemical Pathology Unit of the University College Hospital, Ibadan, Nigeria.

The serum total protein was determined by the Biuret method of Reinhold [24] using a commercial kit (Randox Laboratories Ltd, U.K.), while albumin value was obtained by bromocresol green method of Doumas et al. [8]. The globulin and albumin-globulin ratio were determined according to the method of Coles [4]. The serum creatinine and urea nitrogen were estimated by deproteinization and Urease-Berthelot colourimetric methods respectively, using a commercial kit (Randox Laboratories Ltd., U.K.). Also the serum triglyceride and free cholesterol were determined by nonane extraction and enzymatic colourimetric methods respectively using commercial test kits (Quimica Clinica Applicada, S.A.), while the serum enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were determined using commercial test kits (Randox Laboratories Ltd., U.K.).

Data obtained were subjected to one-way ANOVA procedure of SAS® [27] and the significant treatment means separated by Duncan option of the same software.

RESULTS

The changes in serum proteins of growing pigs fed dietary FB_1 are shown in Figure 1. The animals fed the control and diet 1 had significantly ($P<0.05$) higher serum total protein concentrations than those on diets 2 and 3. The serum total protein concentrations of 81.00 and 88.30 g L^{-1} for the animals on diets 2 and 3, respectively, were about 68 – 74% of those on diet 1 and the control diet at the end of the feeding trial. The mean serum albumin concentrations of the pubertal boars fed diet 1 and the control were significantly ($P<0.05$) higher than the mean serum albumin of those fed diet 2, which was significantly ($P<0.05$) higher than the serum albumin of those on diet 3 containing 15.0 mg FB_1/kg at the end of the feeding trial. The mean serum globulin and albumin-globulin ratio of boars fed diets 2 and 3 were significantly ($P<0.05$) lower

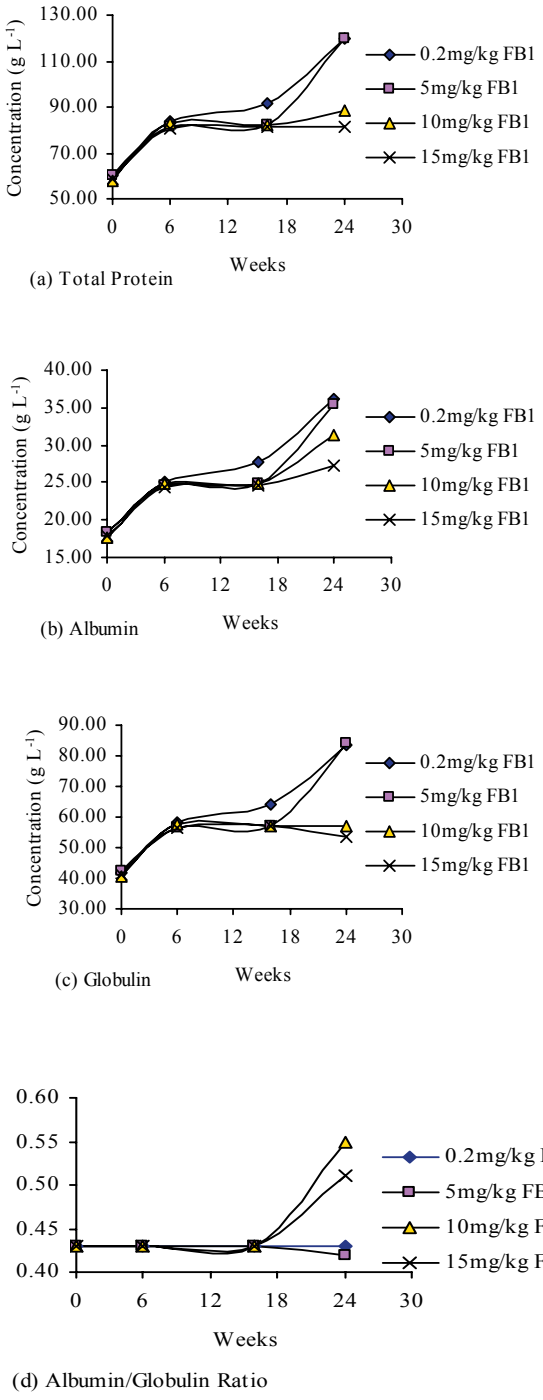


Figure 1: Changes in serum proteins of growing pigs fed varied levels of dietary FB_1

than the mean serum globulin of those fed diet 1 and the control diet at about 33 weeks of age (24 weeks into the feeding trial).

The changes in the serum creatinine shown in Figure 2a revealed time- and dose-dependent increase in the serum creatinine concentrations of the growing pigs, which were not significantly (P>0.05) different among the treatments, while the mean urea nitrogen concentration of the animals on the control diet were lower than the serum urea nitrogen of those fed diets 2 and 3 throughout the experimental feeding period. The mean serum urea nitrogen concentration of the pubertal boars on the control diet was however similar to those fed diets 1, 2 and 3 at the end of the feeding trial.

The serum ALT of the pubertal boars fed diet 2 was significantly (P<0.05) lower than the serum ALT of those fed diet 3 but significantly (P<0.05) higher than the serum ALT of those fed diet 1 and the control (Figure 3a). The serum aspartate aminotransferase (AST) concentrations of growing pigs fed diet 1 and the control were lower than the serum AST of those fed diets 2 and 3 throughout the experimental period (Figure 3b).

Animals fed diets 2 and 3 had significantly (P<0.05) lower serum cholesterol than those fed diet 1 and the control at week 24 of the feeding trial (Figure 4a), while Figure 4b shows that the serum triglyceride concentrations of the animals fed diets 1 and 2 were significantly (P<0.05) lower than the serum triglyceride values of those fed diet 3. A significantly (P<0.05) higher concentration of triglycerides in the group fed diet 2 than those of the control were found. The serum triglyceride of the pubertal boars fed diets 1, 2 and 3 were 105.83, 126.21 and 160.98% of those fed the control diet, respectively.

DISCUSSION

An estimation of the total quantity of serum proteins may be utilized as an estimation of the nutritive state of the animal. The nutritive state may be dependent not only on the proper and adequate intake of protein building materials in the diet but may also be a reflection of the nutritive state existing within the animal body, reflecting

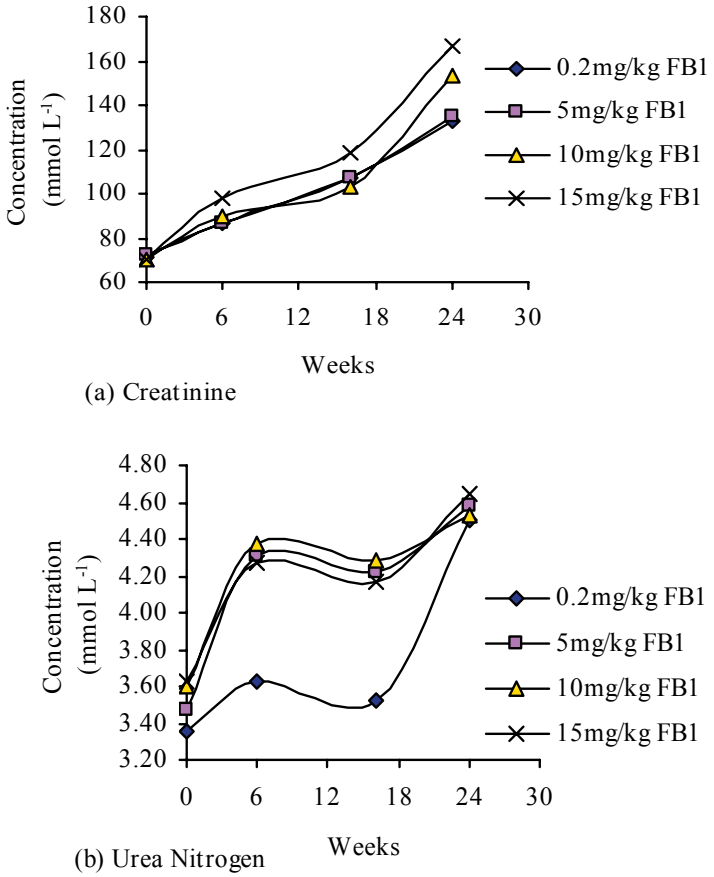
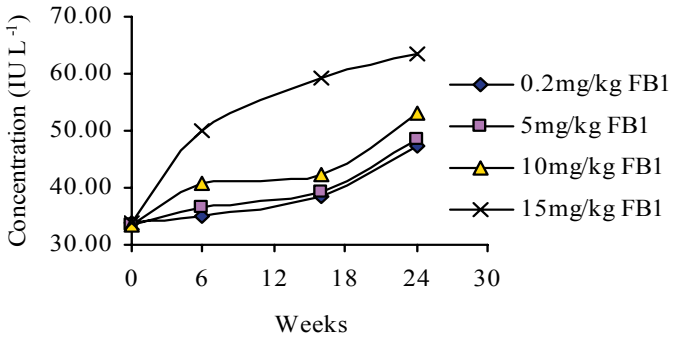


Figure 2: Changes in serum metabolites of growing pigs fed varied dietary FB_1 levels.

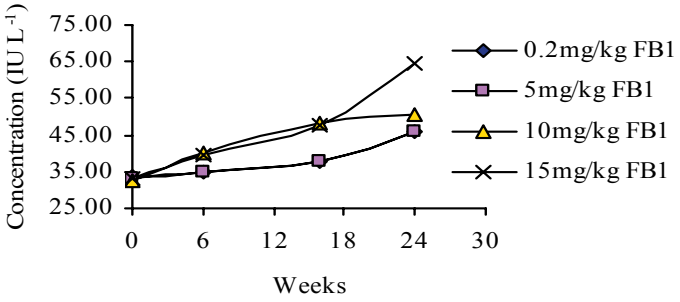
alterations in metabolism. The results obtained in this study showed that the animals fed diets containing ≥10.0 mg FB_1/kg had significantly lower serum total protein and albumin. Hypoproteinemia, as reported by Coles [4], is mostly commonly associated with a lack of proper diet, poor absorption of dietary constituents from the intestinal tract and kidney or liver disease. The results of this study suggest that animals fed diets containing ≥10.0 mg FB_1/kg might have suffered intoxication from altered metabolism as observed by Gbore and Egbunike [10]. Reduced tissue protein synthesis [7] and immunomodulation [29] have been reported to be some of the major symptoms seen in pigs consuming Fusarium mold-contaminated feeds. Since the animals were fed isonitrogenous diets, which contained only varied levels of FB_1, the results revealed the roles which dietary FB_1 could play in serum protein alterations. Coles [4] reported that drastic alterations in serum protein values are often observed in association with both kidney and liver diseases and that an estimation of the serum proteins may be of value both diagnostically and prognostically.

Deficient synthesis of albumin has been reported by Coles [4] to occur mostly in association with chronic hepatic or gastrointestinal diseases in which there is interference with protein digestion and absorption. Bauer et al. [1] reported that a low albumin level might be due to increased loss of albumin in the urine, decreased formation in the liver or insufficient protein intake. The result of the serum albumin obtained suggests that the animals fed diets 2 and 3 might have suffered liver disease since the liver is the sole site of albumin formation and that total proteins may be decreased in animals having liver disease [4]. The serum total protein and albumin values of the pubertal boars fed diets containing ≥10.0 mg FB_1/kg in this study were however within the physiological reference values of 79 – 89 and 18 – 33g L^{-1} reported by Kaneko et al. [15] for the respective parameters for domestic swine. This suggests that the boars fed the control diet and diet 1 did not suffer renal or hepatic disorder.

Bauer et al. [1] reported that a low serum albumin level if continued for any length of time is one of the causes of oedema, since the oncotic pressure is such that water is able to pass from the serum into the tissue space and oedema results. Clinical signs of porcine pulmonary oedema, in which varying amounts of clear yellow fluid accumulate in the pleural cavity, typically occur soon (2 – 7 days) after pigs consume diets (culture material or contaminated maize screenings) containing large



(a) Alanine aminotransferase (ALT)



(b) Aspartate aminotransferase (AST)

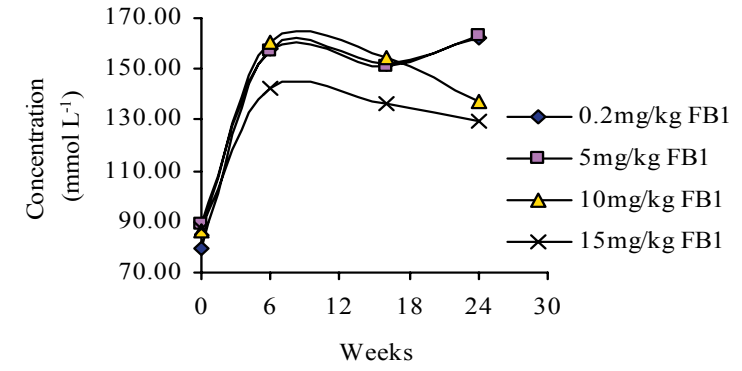
Figure 3: Changes in serum enzymes of growing pigs fed varied levels of dietary FB_1

amounts of fumonisins over a short period of time [31]. The continued low level of serum albumin as a result of dietary FB_1 may be an addition to one of the several hypotheses that have been proposed [5, 14] that contribute to the physiological alteration that results in the inability of the lung to maintain fluid equilibrium.

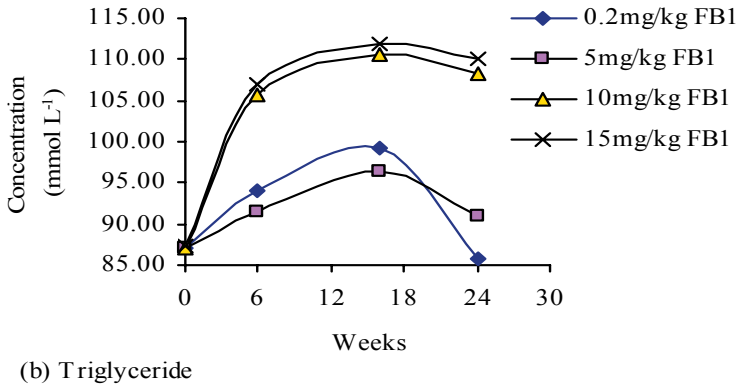
Urea is formed in the liver and represents the principal end product of protein catabolism while creatinine is a metabolic byproduct of muscle metabolism. They are filtered from the blood and excreted in the urine by the kidneys. Coles [4] reported that there is an increase in the serum creatinine and urea nitrogen when there is chronic nephritis (kidney damage). Results revealed that diets containing ≥5.0 mg FB_1/kg significantly increased the serum urea nitrogen compared to those fed the control diet. Coles [4] however reported that serum creatinine determination has a reputation of being a more specific test for the diagnosis and prognosis of progressive renal disease than the serum urea nitrogen, as there are fewer non-renal factors that may influence creatinine. The serum creatinine and urea nitrogen of the experimental boars in this study however were within the physiological reference ranges of 90 – 240 µmol L^{-1} and 3.0 – 8.5 mmol L^{-1} reported by Radostits et al. [23] for the domestic swine for the respective parameters.

Animals on diets 2 and 3 had significantly high activities of the serum ALT throughout the experimental feeding period. The respective serum ALT activities of 59.33 and 63.50 U L^{-1} obtained for the animals on diet 3 (containing the highest FB_1 concentration) at the ends of the pre-pubertal and pubertal growth phases respectively, were above the physiological range values of 31 – 58 U L^{-1} reported by Kaneko et al. [15] for domestic swine. These results suggested that the boars fed diet containing 15 mg FB_1 might have suffered liver disease which caused the liberation of the enzyme into the serum. Champe and Harvey [3] reported that a markedly raised serum activity of serum ALT indicates a severe liver disease, usually viral hepatitis or toxic liver necrosis.

The significantly high values of the serum aspartate aminotransferase (AST) of the animals on diets 2 and 3 throughout the experimental feeding period compared with the serum AST activities of those fed diets containing ≤5.0 mg FB_1/kg suggest that the animals fed diets 2 and 3 might have suffered severe hepatic cell damage which caused the leakage of the enzyme into the extra cellular space. Serum AST, though not as specific to the liver as ALT, has similar role as the ALT. Champe and Harvey



(a) Cholesterol



(b) Triglyceride

Figure 4: Changes in serum lipids of growing pigs fed varied dietary FB_1 levels.

[3] reported that markedly raised serum activities of AST may indicate severe damage to the cells of the liver (as in viral hepatitis or toxic liver necrosis). Although the serum free cholesterol decreased with increased dietary FB_1, the serum triglycerides however increased with the dietary FB_1 concentration. The mechanism underlying this effect of FB_1 on lipid metabolism is unknown; however, multitude changes in liver cholesterol, phospholipids, sphingoid bases and free fatty acid composition created by fumonisins have been reported by Gelderblom et al. [11, 12]. Voss et al. [30] reported significant increases in serum triglycerides, cholesterol and alkaline phosphatase in the male and female Sprague-Dawley rats fed diets containing 0, 15, and 150 mg of FB_1/kg (≥90% pure) for 4 weeks. The authors further reported that the finding confirmed that a dietary level of 150 mg FB_1/kg was hepatotoxic to both sexes.

A study by the National Veterinary Services Laboratory of the US Animal and Health Inspection Agency [20] showed that horses fed 15 mg FB_1/kg in diets formulated from F. proliferatum (M5991) culture material did not exhibit altered serum biochemical parameters after 150 days. However, Wilson et al. [32] reported elevated serum enzyme levels indicative of liver damage in ponies. Also, Motelin et al. [18] reported that increases in free sphinganine in serum paralleled the dose-dependent increases in other biochemical parameters measured at 14 days in pigs.

This study has demonstrated that chronic ingestion of diets containing ≥10 mg FB_1/kg by growing pigs may result in significant alterations in serum protein profiles resulting from chronic hepatic and/ or gastrointestinal diseases.

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